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A novel volume-perturbation calorimeter, which measures time-dependent temperature changes in response to a pressure perturbation, has been used to probe the relaxation dynamics of phospholipid bilayers in the gel to liquid crystalline transition region. The principal relaxation time is between 50 msec to 5 sec. With multilayer systems, a slowing of the relaxation time is observed when the degree of melting is about 0.6. Large unilamellar vesicle relaxation is about an order of magnitude faster than with the multilamellar system. The relaxation dynamics appear to be insensitive to pressure over a pressure range of 10 to 20 atm., thus suggesting that the most important effect of pressure is on the equilibrium properties of the system.

Bilayer-bilayer interactions have been assessed by studies of multilamellar systems in the presence of dextran which dehydrates the interior bilayer-bilayer spaces and forces closer approach between lamellae. Dehydration is accompanied by a reduction in the enthalpy change of about 3 kcal/mole of lipid and produces a broadening of the

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19. transition profile. No such effects are observed in unilamellar vesicles. Monolayer-monolayer interactions have been assessed by use of externally added lathanides to shift the transition temperature of the inner and outer monolayers of unilamellar vesicles. Complete separation of the melting of the two monolayer can be achieved, indicating that monolayer-monolayer coupling is small.

The effects of hydrostatic pressure on these interactions are being investigated using a variable pressure DSC. A new cell has been constructed and tested. The effect of pressure of the transition of pure multilamellar liposomes is to increase the transition temperature with no change in the enthalpy or shape of the heat capacity curve. The magnitude of the shift is quantitatively predicted from the Clausius-Claperyon equation. In mixed lipid systems, however, the effect of presure is more complex.

Monte Carlo techniques are being employed to quantitatively model the equilibrium properties of the system and to describe some simple dynamic properties (e.g. diffusion) of the system.

Annual Report on
"The Effect of Moderate
Pressures on Biological Processes"

N00014-88-K-0326

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I. Introduction

The effect of pressure on an equilibrium process is manifested via the volume change of the process. In order for moderate pressure (50 to 100 atm.) to produce a significant effect, the associated volume change must be substantial ($\geq$ 250 cm^3/mole). Such large volume changes will most likely only occur for cooperative processes involving many molecules. A most likely source of such volume changes in biological systems are concerted structural changes in the lipid matrix of a membrane, an example of which is the gel to liquid crystalline transition. If such a transition is coupled, for example, to a protein function, then pressure could greatly alter the physiological functioning of the system. In the past year we have initiated studies of the effect of pressure on these transition characteristics. These studies include the effect of pressure on the thermodynamics of the transitions and the dynamics of the transitions and a dissection of specific lipid-lipid interactions which are important in defining these properties. Also, we have initiated Monte Carlo studies in an attempt to describe thermodynamic and dynamic properties of these systems in some molecular

detail.

II. The effect of Pressure on the Thermotropic Phase Behavior of Pure Lipid Bilayers.

A new pressure cell for our differential scanning calorimeter (1) has been designed and constructed. With this cell, the hydrostatic pressure can be increased by helium gas up to 2000 psi and maintained constant throughout an experiment. The aqueous dispersion of lipid is contained in the bottom of the calorimetric cell and covered by polyethylene disc to prevent evaporation. The data obtained with new pressure cell using dipalmitoylphosphatidylcholine liposomes are identical to those previously obtained (2), but is of higher quality. The precision in determining the melting temperature is ± .05°C and the pressure dependence of the T_m on pressure is 0.025°/atm. This result is predicted by the Clausius-Claperyon equation: $dT_m/dp = \Delta V/\Delta S$. No effect on the shape of the heat capacity curve or the enthalpy change occurs.

The improved cell design was necessary to obtain the complete heat capacity profile for mixed lipid systems which can melt over a 30 to 60 degree temperature range. Preliminary results with mixed lipid systems indicate that pressure greatly effects the thermodynamic behavior in the two phase-region suggesting large pressure effects on the interaction between liquid and gel phases at the interfacial region.

III. A Volume Perturbation, Dynamic Calorimeter

We have written a review (3) describing the theoretical basis of a dynamic

calorimeter for measuring the relaxation kinetics of reactions and transitions which involve volume changes. The instrument uses a stack of piezo-electric crystals to induce small, adiabatic volume oscillations in a sample, thereby perturbing the equilibrium. Relaxation of a system to the time-dependent equilibrium is observed by monitoring its temperature and pressure over time. Data collected from a sample under study are analyzed in the frequency domain after they have been corrected for the response characteristics of the instrument and aqueous medium of the sample. Relaxation times are obtained by normal mode analysis using a nonlinear least-squares fitting algorithm.

This instrument has been used to study the kinetics of the main phase transition in (i) one-component multilamellar vesicles of phosphatidylcholine, with acyl or alkyl chains, and of phosphatidyl serine; (ii) in one-component large unilamellar vesicles of DMPC or DPPC; and (iii) in two-component multilamellar dispersions of DPPC and either the local anesthetic dibucaine or the general anesthetic 1-dodecanol. The relaxations in all these systems are characterized by a single relaxation time, which is greatly enhanced in a neighborhood of the transition temperature (T_m). The slowest rate of relaxation occurs at a temperature slightly higher than T_m. The presence of dibucaine reduces the level of enhancement of the relaxation time, but this effect saturates at a nominal mole ratio of lipid to anesthetic of 150/1. The degree of enhancement of the relaxation time depends strongly on the nature of the lipid headgroup and the nature of the bonding of the hydrocarbon chains to glycerol. The kinetic models of Schwarz (4) and Kanehisa and Tsong (5) for cooperative melting are inadequate for explaining the observed relaxations.

IV. Studies on Bilayer-Bilayer and Monolayer-Monolayer Interactions

Bilayer vesicles of phospholipids can exist in either single or multilamellar form depending on the method of preparation. The various types of molecular interactions that can influence the equilibrium behavior of these systems include lipid-lipid interaction within the monolayer, interactions between monolayers within a single bilayer and interactions between bilayers in multilamellar systems. If one is to investigate the effect of pressure on the thermodynamics and kinetics of phase transitions of these systems, it is necessary to obtain estimates of the significance of these types of interactions. This is particularly true in light of our hypothesis that it is the lipid-lipid interactions within a monolayer that are important in coupling proteins with lipid structural changes.

Monolayer-monolayer interactions were investigated by assessing the effect of lathanide on the transition of DPPC vesicles. The lathanide, when added to the outside of the vesicle, should only influence the outside monolayer, raising its melting temperature as previously shown by NMR experiments (6). This has been found to be true using DSC to monitor the transition. Our results are consistent with all monolayers melting independently. That is, there is no significant coupling across the monolayer interface.

The significance of bilayer-bilayer interactions has been probed by using multilamellar systems to which dextran has been externally added. This procedure produces an osmotic gradient between the external medium and the bilayer interstitial spaces which is relieved by dehydration of the spaces thus reducing bilayer-bilayer distance. Our results clearly show that when the distance is reduced to less than 25 Å, as described by Parsegian and coworkers (7), a significant reduction in the enthalpy change occurs with concomitant broadening

of the heat capacity curve. Detailed analysis of the dextran concentration dependence, however, indicated that at distances normally assumed (i.e. in the absence of dextran) in multilamellar structures, this interaction is small.

The above studies indicate that pressure effects on the thermotropic behavior of DPPC liposomes will primarily be the reflection of the volume change associated with the transition or the effect of pressure on lipid-lipid interactions within a single monolayer.

V. Monte Carlo Studies

Monte Carlo studies to describe the equilibrium and dynamic properties of bilayers have been initiated. The approach is similar to that previously described by Freire and Snyder (8) and Zuckerman and Mouritson (9). Of particular interest are results obtained for simple diffusion models where observed diffusion is extremely sensitive to the details of lipid-lipid interactions. The results suggest that pressure studies on lateral diffusion in conjunction with pressure studies on the thermotropic behavior of lipid bilayers could be very useful.

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